SUN PROTECTION FACTOR EVALUATION OF PREPARED HERBAL SUNSCREEN GELS FROM *PREMNA INTEGRIFOLIA* L. (TAUNG-TAN-GYI) STEM

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Abstract

The present study focused on the preparation of herbal sunscreen gels from *P. integrifolia* stems. The qualitative preliminary phytochemical investigation was carried out by the reported chemical methods. The prepared herbal sunscreen gels by using watery and ethanol extracts were examined for evaluation parameters such as colour, odour, appearance, consistency, homogeneity, washability, pH, viscosity and sun protection factor (SPF). The prepared herbal sunscreen gels had a pleasant odour, smooth appearance, acceptable consistency, great homogeneity, ease of removal and brown for watery extract and yellowish brown for ethanol extract containing prepared gels. The pH of prepared gels was near skin pH, found to be 6.3 and 6.2 for watery and ethanol extracts containing gels. The viscosity results indicated that both of the two extracts using gels had > 5000 cP revealing the easy spreadability of gel application on the skin. The *in vitro* SPF values were found to be 16.16 and 13.89 for watery and ethanol extracts containing prepared gels, respectively, compared to marketed products (34.48) by using the UV spectrophotometric method. *In vitro* screening of antibacterial activity showed a mild antibacterial effect against *Staphylococcus aureus* by the agar well diffusion method. Skin irritation tests exhibited no redness, edema, inflammation, or irritation in 10 volunteers (male/female) using patch tests. The prepared herbal sunscreen gels containing watery and ethanol extracts from *P. integrifolia* stem were effectively utilized as sunscreen with significant sun protection properties and were safe with respect to skin irritation and allergic sensitization. The result can form the basis for the development of novel broad-spectrum sunscreen formulations.

Keywords: antibacterial activity, herbal sunscreen gels, *Premna integrifolia* L., skin irritation test, SPF

Introduction

Sunlight is composed of various wavelengths ranging from ultraviolet light through infrared to visible light. The solar spectrum radiation of the sun is divided into five regions: Ultraviolet C or UV-C (from 100 nm to 290 nm), Ultraviolet B or UV-B (from 290 nm to 320 nm), Ultraviolet A or UV-A (from 320 nm to 400 nm), visible range or light (from 380 nm to 780 nm) and infrared (from 780 nm to 10^6 nm) (Mamillapalli *et al*., 2018). Overcome the bad effects of ultraviolet rays or sunlight, one of which is using sunscreen. This sunscreen is a cosmetic ingredient that physically and real or chemically can inhibit and penetrate UV light into the skin (Yusnelti *et al*., 2018). A sunscreen is a photoprotective agent against direct UV radiation and is used as the skin's defense against the harmful effects of UV radiation. Its ability is to absorb or reflect the sun's UV radiation on the skin from over exposure to UV radiation. They help to prevent sunburn, skin damage and skin cancer (Jangde and Daharwal, 2011). Synthetic photoprotective agents can be potentially toxic and carcinogenic and therefore phytoconstituents are becoming popular as essential ingredients of cosmetic formulations (Eff *et al*., 2019). Today people are showing more interest towards herbal cosmetics comparatively

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synthetic cosmetic products. The best thing about herbal cosmetics is that they are purely made from herbs and shrubs, so they are side-effect free and free from harmful chemicals. The main benefits reported for herbals, used in skincare and cosmetics include antioxidant, anti-inflammatory, antiseptic, and antimicrobial properties (Roy and Sahu, 2014).

Premna integrifolia L. (Figure 1) (Taung-tan-gyi in Myanmar) belongs to the Verbenaceae family and it comprises 200 species of which 30 are available in India. Its hypolipidemic, anti-diabetic, anti-inflammatory and immuno-modulatory activities were reported. Various parts of plants, like leaves, stem, stem barks, root, root barks and wood are used to treat different diseases (Chitra *et al*., 2018). To improve the sun protection activity of the prepared herbal sunscreen gels, the incorporation of watery and ethanol extracts of *P. integrifolia* stem was conducted according to Grace *et al.* (2014) with some modifications.

Botanical Description of *Premna integrifolia* L. (Taung-tan-gyi)

Family name	: Verbenaceae
Genus	: *Premna*
Specie	: *integrifolia*
Botanical name	: *Premna integrifolia* L. (Kress *et al*., 2003)
Myanmar name	: Taung-tan-gyi
Part used	: Stem
Distribution	: the plant prefers warm and humid climate

Figure 1. Photograph of *Premna integrifolia* L.

Materials and Methods

Sample Collection and Identification

In this research, the stem of *P. integrifolia* was collected from a local market in Tanintharyi Region, Myanmar. After cleaning, the sample was air-dried at room temperature for one week and the dried sample was ground into powder by a grinder. The dried powdered sample was stored separately in air-tight containers to prevent moisture changes and other contamination. The sample was identified by an authorized botanist of the Department of Biology, Sagaing University of Education, Myanmar.

Preliminary Phytochemical Tests

The preliminary phytochemical tests of the selected sample were carried out using the standard methods (Harborne, 1984; Marini-Bettoto *et al*., 1981 and M-Tin Wa,1972).

Preparation of Extracts

After performing the preliminary phytochemical tests of the selected sample, it is required to obtain watery and ethanol extracts for the preparation of herbal sunscreen gels according to the reported methods.

Preparation of Herbal Sunscreen Gels

Herbal sunscreen gels were prepared according to Grace *et al*. (2014) with some modifications. For 100 g herbal sunscreen gel, the preservatives methylparaben (0.1 %) and propylparaben (0.1 %) were dissolved in a few mL of distilled water and the gelling agent carbopol 940 (2 %) was added to the above solution. Then the solution was kept under constant stirring using a magnetic stirrer for complete dissolution of the contents. After complete dissolution, humectant glycerine (2 %) and neutralizer triethanolamine (2 %) were added under constant stirring until a uniform mixture. The active ingredient extract (1 %) and sufficient amount of distilled water were then carefully added to the above mixture and stirred continuously until a smooth and homogeneous gel was obtained.

Evaluation of some Parameters of Herbal Sunscreen Gels

The colour and odour of the samples were evaluated both visually and manually. The appearance of the samples was judged by their colour, roughness, and grade.

The consistency of the samples was evaluated by manually. The homogeneity of the samples was evaluated by visual appearance and touch. The washability of the samples was applied to the hand and washed by keeping the hand with tap water.

About 0.5 g of the sample was weighed and dispersed in 50 mL of distilled water to determine the pH using the pH paper and pH meter (Model HI-9812 HANNA instruments). The viscosity of the sample was measured using a rotor spin type (L-4) at 100 rpm with an ATAGO viscometer (BASE Series L, Japan) in Physical Research Room, Department of Chemistry, University of Yangon. The viscosity values of the samples were recorded directly from the instrument display.

Determination of Sun Protection Factor (SPF)

Determination of SPF of prepared herbal sunscreen gels was carried out by UV-vis spectrophotometer and Mansur mathematical calculation method at the Medical Biotechnology Laboratory, Department of Biotechnology Research (DBR), Kyaukse Township, Mandalay Region, Myanmar.

The photoprotective activity of tested samples was measured by the determination of sun protection factor (SPF) (Mansur *et al*., 1986). Sample (2 mg) was diluted in 1 mL of methanol to obtain a concentration of 2 mg/mL. Spectrophotometric scanning was performed at wavelengths between 290 and 320 nm, with intervals of 5 nm using a 96-well microplate reader. SPF value was obtained according to the equation developed by (Mansur *et al*. 1986) and Kumar *et al*. (2016). The SPF value was calculated using the following equation.

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

CF = correction factor (= 10)
EE (λ) = erythemogenic effect of radiation with wavelength λ
I (λ) = solar intensity spectrum
Abs (λ) = spectrophotometric absorbance values of sunscreen product at wavelength λ
EE × I = constant is determined by Sayre *et al*. (1979) **Skin Irritancy Test on Human**

Skin

The skin irritancy test was performed on 10 selected adults (male/female) from Sagaing University of Education, Myanmar. According to the patch test description, the student volunteers were divided into two groups. Each group contained five student volunteers who were physically and mentally healthy, skin free from lesions and no history of allergies. Groups (A) and (B) volunteers were marked with an area of 3 cm^2 on their left-hand dorsal surface applied with 0.5 g prepared herbal sunscreen gel containing watery and ethanol extracts and their right-hand dorsal surface was used as control (without applied gels). Then, irritancy, erythema, and edema were checked if any, at regular intervals up to every 24 h for fourteen days, and irritation reactions were recorded.

Investigation of Antibacterial Activity

Screening of the antibacterial activity of prepared herbal sunscreen gels were carried out against Gram-positive bacteria (*Staphylococcus aureus*) as the tested microorganism for this experiment. The tests were screened at the Pharmaceutical Research Laboratory, Department of Biotechnology Research (DBR), Kyaukse Township, Mandalay Region, Myanmar.

The agar well diffusion method was used for antibacterial activity evaluation by modifying the previous method (Schlegel and Zaborosch, 1993). Tested microorganisms were inoculated in Muller Hinton Broth at 37 ˚C for overnight. On the next day, the overnight broth culture was diluted with normal saline to obtain the OD_{600} at 0.08 to 0.1 with an approximate cell density of 1.5×10^8 CFU/mL. Muller Hinton agar plates were prepared and sterilized by autoclaving at 121 ˚C for 15 min. The broth inoculums were evenly spread out with sterile cotton swabs on the Muller Hinton agar plates to obtain the uniform inoculums. After the plate was inoculated, 8 mm diameter wells were made on the agar medium by using a sterile cork borer. Each 50 µL (0.2 g/mL) of samples was introduced into each labelled well. Chloramphenicol (30 µg/well) was used as the positive control. Then, the plates were placed in an incubator at 37 ˚C for 16 to 18 h. After incubation, the plates were examined and zone diameters of complete inhibition were measured and recorded to the nearest millimeter.

Results and Discussion

Phytochemicals Present in the *P. integrifolia* Stem

The qualitative preliminary phytochemical screening of *P. integrifolia* stem revealed the presence of alkaloids, amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins and starch in the sample. However, steroids and tannins were not detected in the sample (Table 1). Therefore, to improve the sun protection activity of prepared herbal sunscreen gels, a good combination of phytoconstituents was conducted, and thus confirming the SPF value in this research.

Table 1. Results of Phytochemical Investigations of *Premna integrifolia* Stem

No	Test	Extract	Test reagent	Observation	Result
1	alkaloids	1% HCl	Dragendorff's reagent Mayer's reagent Wagner's reagent	orange ppt. white ppt. reddish brown ppt.	+ + +
2	α-Amino acids	H_2O	Ninhydrin reagent	violet colour	+
3	carbohydrates	H_2O	10 % α-Naphthol, conc. H_2SO_4	red ring	+
4	flavonoids	EtOH	Mg turning, conc. HCl	pink colour	+
5	glycosides	H_2O	10 % lead acetate	white ppt.	+
6	phenolic compounds	EtOH	1 % $FeCl_3$ solution	dark blue colour	+
7	reducing sugars	H_2O	Benedict's solution	brick-red ppt.	+
8	saponins	H_2O	distilled water	frothing	+
9	starch	H_2O	1 % iodine solution	blackish blue	+
10	steroids	PE	acetic anhydride, conc. H_2SO_4	no greenish blue	−
11	tannins	H_2O	1 % gelatin	no white ppt.	−

(+) = presence, (−) = absence, (ppt.) = precipitate

Herbal Sunscreen Gels Prepared from Plant Extracts

After performing the preliminary phytochemical tests, it is required to obtain watery and ethanol extracts for the preparation of herbal sunscreen gels. Plant extracts containing a wide range of phenolic acids, flavonoids and high molecular weight polyphenols usually cover the full range of UV wavelengths. These bioactive components have the capability to absorb UV radiation (Bambal *et al*., 2011). To improve the sun protection activity of prepared herbal sunscreen gels, the incorporation of watery and ethanol extracts of *P. integrifolia* stem was conducted.

Some Parameters of Herbal Sunscreen Gels

The observed colour of the prepared herbal sunscreen gel containing watery extract was brown, and the ethanol extract was yellowish brown. Both of the prepared gels had a pleasant odour.

The prepared herbal sunscreen gels had smooth and good consistency and had no gritty particles. The homogeneity test confirms the uniform distribution of extracts in the prepared gels and shows the absence of granules. So, from the prepared gels, it can be said that they had great homogeneity. The prepared herbal sunscreen gels exhibited a non-greasy effect, after application to the skin. The gels were easily removed by washing them with tap water.

The pH of the prepared herbal sunscreen gels containing watery extract was found to be 6.3 and 6.2 for ethanol extract. These pH values agree with the specification (4.5-8.0) of the

Sunscreen Preparations National Standardization Agency (SNI, 1996), corresponding to the human skin pH (4.5-7.0). So, the prepared herbal sunscreen gels were safe to use on the skin. The viscosity of the prepared herbal sunscreen gels was found to be > 5000 cP, meeting the requirements of sunscreen preparations (2000-50000 cP) (SNI, 1996).

Sun Protection Factor of the Prepared Herbal Sunscreen Gel

The results of the SPF determination of the prepared herbal sunscreen gels indicated that watery extract possesses a higher SPF value 16.16 than ethanol extract 13.89. The SPF value of the prepared sample is very appreciative when it is compared with that of other herbal extracts reported by various authors (Bambal *et al*., 2011; Mamillapalli *et al*., 2018; Yanti Eff *et al*., 2019). The SPF of the standard marketed product was found to be 34.48. The prepared herbal sunscreen gels were found to have sun protection activity of 94 % for watery extract and 93 % for ethanol extract. Thus, prepared gels exhibit significant sun protection activities and may be effectively utilized as herbal sunscreen gels preferred by users with oily skin (Tables 2, 3, 4, 5 and Figure 2).

Table 2. SPF Value of Prepared Herbal Sunscreen Gel-H_2O

Sample	Wavelength	Average Absorbance	EE × I	Abs × (EE × I)
Gel-H_2O	290	1.96 ± 0.21	0.0150	0.0294
	295	1.85 ± 0.05	0.0817	0.1511
	300	1.78 ± 0.01	0.2874	0.5116
	305	1.68 ± 0.01	0.3278	0.5507
	310	1.32 ± 0.01	0.1864	0.2460
	315	1.26 ± 0.01	0.0839	0.1057
	320	1.19 ± 0.01	0.0180	0.0214
				1.6159 ± 0.14

$$\mathbf{SPF} = \mathbf{CF} \times \sum_{290}^{320} \mathbf{.\ EE\ (\lambda) \times I\ (\lambda) \times Abs\ (\lambda)}$$

$$= 10 \times 1.6159$$

$$= 16.16$$

Table 3. SPF Value of Prepared Herbal Sunscreen Gel-EtOH

Sample	Wavelength (nm)	Average Absorbance	EE × I	Abs × (EE × I)
Gel-EtOH	290	2.28 ± 0.20	0.0150	0.0342
	295	1.96 ± 0.02	0.0817	0.1601
	300	1.78 ± 0.02	0.2874	0.5116
	305	1.25 ± 0.02	0.3278	0.4098
	310	1.13 ± 0.02	0.1864	0.2106
	315	0.64 ± 0.02	0.0839	0.0537
	320	0.51 ± 0.01	0.0180	0.0092
				1.3892 ± 0.18

$$\mathbf{SPF} = \mathbf{CF} \times \sum_{290}^{320} \mathbf{.\ EE\ (\lambda) \times I\ (\lambda) \times Abs\ (\lambda)}$$

$$= 10 \times 1.3892$$

$$= 13.89$$

Table 4. SPF Value of Marketed Product

Sample	Wavelength(nm)	Average Absorbance	EE × I	Abs × (EE × I)
sunblock 27 from Nature Republic (Korea)	290	3.49 ± 0.01	0.0150	0.0524
	295	3.50 ± 0.00	0.0817	0.2860
	300	3.49 ± 0.03	0.2874	1.0030
	305	3.50 ± 0.00	0.3278	1.1473
	310	3.41 ± 0.09	0.1864	0.6356
	315	3.16 ± 0.11	0.0839	0.2819
	320	3.36 ± 0.13	0.0180	0.0569
				3.4482 ± 0.26

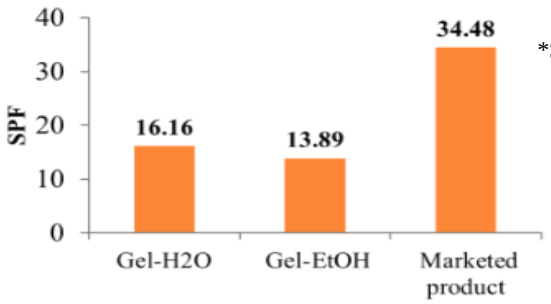
$$\mathbf{SPF} = \mathbf{CF} \times \sum_{290}^{320} \mathbf{.\ EE\ (\lambda) \times I\ (\lambda) \times Abs\ (\lambda)}$$

$$= 10 \times 3.4482$$

$$= 34.48$$

Table 5. Comparison of SPF Values of Prepared Gels and Marketed Product

No	Sample	SPF Calculated Value	UV Protection Rate (%)
1	Gel-H_2O	16.16	94
2	Gel-EtOH	13.89	93
3	*Marketed product	34.48	97



*Sunblock 27 from Nature Republic (Korea)
SPF (Reference): 2-12 = minimum sun protective activity
12-30 = moderate sun protective activity
≥ 30 = high sun protective activity
≥ 50 = very high sun protective activity
(Ratnasooriya *et al*., 2014)

Figure 2. Comparison of SPF values of prepared gels and *marketed product

Irritancy Test on Human Skin

Evaluation of the efficacy of sunscreen for a long time has been assessed through skin test, which was performed with human volunteers. After two weeks of the applied part with prepared gels, a skin irritation study exhibited no redness, edema, inflammation, irritation, sensitivity, or minor or patchy erythema. The prepared herbal sunscreen gels were safe to use on the skin and provided complete protection from skin irritation.

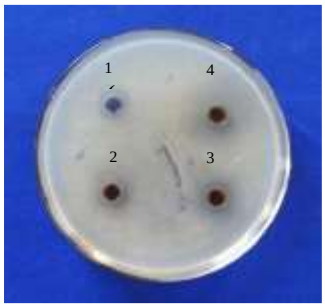
Antibacterial Activity of Prepared Herbal Sunscreen Gels

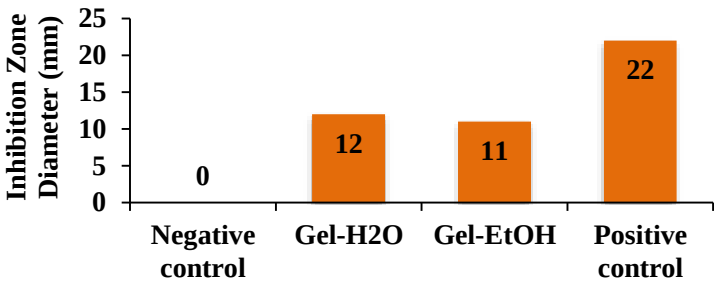
The prepared gel showed only mild antibacterial activity on *S. aureus* (Table 6 and Figure 3). *S. aureus* mainly causes skin infections and thrives best at pH 7.5, while at pH 5 to 6, they grow poorly (Tiwari *et al*., 2022). The pH values of prepared gels were found to be 6.3 for watery extract and 6.2 for ethanol extract. The results of the pH determination indicated an appropriate pH range for inhibition of *S. aureus* skin infection.

Table 6. Antibacterial Activity of Prepared Herbal Sunscreen Gels on *S. aureus*

No.	Sample	Inhibition zone diameters (mm)
1	70 % EtOH	0
2	Gel-H_2O	12
3	Gel-EtOH	11
4	Chloramphenicol	22

Agar well = 10 mm | 10 mm ~ 14 mm Activity weak | 15 mm ~ 19 mm high activity | 20 mm above very high activity





1 = Negative control (70 % EtOH)
2 = Herbal sunscreen cream-H_2O extract
3 = Herbal sunscreen cream-EtOH extract
4 = Positive control (Chloramphenicol)

Figure 3. Antibacterial activity of prepared gels on *S. aureus*

Conclusion

Herbal sunscreen gels were successfully prepared using watery and ethanol extracts from *Premna integrifolia* L. (Taung-tan-gyi) stem in this research. From the results obtained in the study, it can be concluded that the prepared herbal sunscreen gels meet the requirements for sunscreen evaluation parameters such as colour, odour, appearance, consistency, homogeneity, washability, pH, viscosity and SPF value. In addition, the prepared herbal sunscreen gels did not irritate the skin, according to the antibacterial activity investigation and skin irritancy study. Incorporating herbal extracts into sunscreen gel preparation, SPF values showed 16.16 (94 % sun protection activity) for watery extract and 13.89 (93 % sun protection activity) for ethanol extract, which were found to have not much different sun protection properties in this research.

Acknowledgements

The authors would like to express their profound gratitude to the Department of Higher Education, Ministry of Education, Myanmar, Pro-Rectors Dr Khin Hnin Yee and Dr Cho Kyi Than (Sagaing University of Education) for provision of opportunity to do this research. Special thanks are extended to the specialists from Medical Biotechnology Laboratory, Department of Biotechnology Research (DBR), Kyaukse, Mandalay Province, Myanmar and Department of Chemistry, University of Yangon for their cooperation.

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